12-27-99

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	4121-115
	U S APPLICATION NO (If known, see 37 CFR 1 5) 09/446808

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE98/01797	24 June 1999	24 June 1997

TITLE OF INVENTION

Mammal with Inhibition of the Poly (ADP Ribose) Polymerase and Method for Using Same to Identify Cancerigenic Agents

APPLICANT(S) FOR DO/EO/U.S. Patent and Trademark Office

KUPPER, Jan-Heiner; BURKLE, Alexander; van GOOL, Leon; and zur HAUSEN, Harald

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C 371
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U S C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b ☐ has been transmitted by the International Bureau
   c ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U S C 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S C 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) *
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1 98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A small entity statement (independent inventor statement)
16. ☐ Other items or information:

NOTE: This application is being filed without an Oath or Declaration under the provisions of 37 CFR § 1 53 in order that applicants may secure a filing date of December 23, 1999 Upon receipt of a "Notice of Missing Requirements," a Declaration and Power of Attorney and a Small Entity Statement, will be filed in the Patent and Trademark Office. The undersigned attorney affirmatively states that he has been duly authorized and appointed to file this application on behalf of the applicants and applicants' assignee, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment An appertaining Small Entity Statement will also be submitted for such assignee in response to a Notice of Missing Requirements.

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)). Search Report has been prepared by the EPO or JPO $970 00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $0.00		
No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $0.00		
Neither international preliminary examination fee (37 CFR 1 482) nor international search fee (37 CFR 1 445(a)(2)) paid to USPTO $1,070.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $0.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 970.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1 492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20-11 =	0	X **$18.00**	$ 0.00	
Independent Claims	3-2 =	0	X **$78.00**	$ 0.00	
Multiple dependent claim(s) (if applicable)			+ **$260.00**	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 970.00	
Reduction by 1/2 for filing by small entity, if applicable Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28)				$ -485.00	
SUBTOTAL =				$ 485.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$ 0.00	
TOTAL NATIONAL FEE =				$ 485.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+	$ 0.00	
TOTAL FEE ENCLOSED =				$ 485.00	
				Amount to be refunded:	$
				Charged	$

a. ☒ A check (IPTL Check No. 6575) in the amount of **$485.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist
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P. O. Box 14329
Research Triangle Park, NC 27709

William A. Barrett
Registration No. 42,296

4121-115
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:	
Applicants:	KUPPER et al.
Serial No.:	New National Phase U.S. Patent Application Claiming Priority to PCT International Application No. PCT/DE98/01797
International Filing Date:	24 June 1998
Priority Date Claimed:	24 June 1997 (German Application No. 197 26 702.5)
Title of International Application:	A Method for Identifying Cancerigenic Agents Using a Mammal With Inhibition of the Poly (ADP Ribose) Polymerase
Title as Amended by Preliminary Amendment Submitted Herewith:	Mammal with Inhibition of the Poly (ADP Ribose) Polymerase and Method for Using Same to Identify Cancerigenic Agents

EXPRESS MAIL CERTIFICATE

It hereby is certified by the person identified below that the attached documents are being mailed by such person to the Assistant Commissioner of Patents and Trademarks on the date specified, in an envelope addressed to the Assistant Commissioner of Patents, Box PATENT APPLICATION, Washington, DC 20231, and Express Mailed under the provisions of 37 CFR 1.10.

Jacqueline Bagwell

December 23, 1999
Date of Mailing

EL387762973US
Express Mail Label Number

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

In the Title:

Replace "A Method for Identifying Cancerigenic Agents Using a Mammal With Inhibition of the Poly (ADP Ribose) Polymerase" with --Mammal with Inhibition of the Poly (ADP Ribose) Polymerase and Method for Using Same to Identify Cancerigenic Agents--.

In the Claims:

Cancel claims 1 and 7-9.

In claim 2, replace "2)" with --2.--, replace "process" with --method-- and replace "1" with --10--.

In claim 3, replace "3)" with --3.--, replace "process" with --method-- and replace "1" with --10--.

In claim 4, replace "4)" with --4.--, replace "process" with --method-- and replace "1" with --10--.

In claim 5, replace "5)" with --5.--, replace "process" with --method-- and replace "1" with --10--.

In claim 6, replace "6)" with --6.--, replace "process" with --method-- and replace "1" with --10--.

Add the following new claims 10-15.

10. A method for identifying cancerogenic agents, said method comprising the step of administering one or more potentially carcenogenic agents to a mammal having a genome comprising a DNA repair disturbance caused by inhibiting the poly(ADP ribose)polymerase.

11. A transgenic mammal comprising a genome comprising a DNA repair disturbance caused by inhibiting the poly(ADP ribose)polymerase.

12. The transgenic mammal of claim 11 wherein the mammal is a mouse.

13. The transgenic mammal of claim 11 wherein the inhibition of the poly(ADP-ribose)polymerase is caused by expression of a dominant negative poly(ADP ribose)polymerase.

14. The transgenic mammal of claim 11 wherein the inhibition of the poly(ADP-ribose)polymerase is made by a transgenic operation.

15. The transgenic mammal of claim 11 wherein the genome of the mammal comprises the DNA construct shown in Figure 1.

REMARKS

Claims 1 and 7-9 have been cancelled and claims 10-15 have been added. It is therefore requested that the examination and prosecution of this application proceed on the basis of claims 2-6 and 10-15.

The fees payable for the entry of these claims have been calculated on the Transmittal Letter submitted herewith. Please charge any additional fees necessary to the entry of this amendment and/or the filing of the accompanying application, and credit any excess, to Deposit Account No. 08-3284 of Intellectual Property/Technology Law.

Respectfully submitted,

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Attorney for Applicants

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

23448

PATENT TRADEMARK OFFICE

5001

In re United States Patent Application of:

Applicant: KÜPPER, et al.

Application No: 09/446,808

Examiner: Not Yet Assigned

Group Art Unit: Not-Yet Assigned

Date Filed: 23 December 1999

Title: MAMMAL WITH INHIBITION OF THE POLY (ADP RIBOSE) POLYMERASE AND METHOD FOR USING SAME TO IDENTIFY CANCERIGENIC AGENTS

~~3700~~ (5640)

EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Assistant Commissioner for Patents on the date specified below, in an Express Mail envelope addressed to the Assistant Commissioner for Patents, Box PCT, Washington, DC 20231, and First Class Mailed under the provisions of 37 CFR 1.10.

Lynda Montoya

April 25, 2000

Date of Mailing

EL484453746US

Express Mail Certificate

SUBMISSION OF VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(e)4 & 1.27(d)) - - FOREIGN NON PROFIT ORGANIZATION

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

I hereby declare that I am:

☐ the owner of the foreign non-profit organization identified below:

☒ an official of the foreign non-profit organization empowered to act on behalf of the concern identified below:

NAME OF NON-PROFIT ORGANIZATION: **DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DES ÖFFENTLICHEN RECHTS**

ADDRESS OF NON-PROFIT ORGANIZATION: **Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany**

I hereby declare that the above identified foreign non-profit organization qualifies as a non-profit organization as defined in 37 CFR 1.9(e), for purposes of paying reduced fees to the United States Patent and Trademark Office.

I hereby aver that exclusive rights under contract or law have been conveyed to and remain with the foreign non-profit organization identified above with regard to the invention described in the above-referenced U.S. Patent Application.

If the rights held by the above-identified non-profit organization are not exclusive, each individual, concern, or organization having rights in the invention must file separate verified statements averring to their status as small entities, and that no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a non-profit organization under 37 CFR 1.9(e). No such person, concern, or organization exists.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at any time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR 1.28(b).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Prof. Dr. med. H. zur Hausen Dr. rer. pol. J. Puchta

TITLE OF PERSON IF OTHER THAN OWNER Wiss. Stiftungs-stand Adm. Stiftungsvorstand

ADDRESS OF PERSON SIGNING Eichenstr.1,69483 Wald-michelbach Eichenweg 12a, 69198 Schries-heim

SIGNATURE _____ DATE 29.02.2000

NAME OF NON-PROFIT ORGANIZATION: **DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DES ÖFFENTLICHEN RECHTS**

ADDRESS OF NON-PROFIT ORGANIZATION: **Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany**

I hereby declare that the above identified foreign non-profit organization qualifies as a non-profit organization as defined in 37 CFR 1.9(e), for purposes of paying reduced fees to the United States Patent and Trademark Office.

I hereby aver that exclusive rights under contract or law have been conveyed to and remain with the foreign non-profit organization identified above with regard to the invention described in the above-referenced U.S. Patent Application.

If the rights held by the above-identified non-profit organization are not exclusive, each individual, concern, or organization having rights in the invention must file separate verified statements averring to their status as small entities, and that no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a non-profit organization under 37 CFR 1.9(e). No such person, concern, or organization exists.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at any time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR 1.28(b).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Prof. Dr. med. H. zur Hausen Dr. rer. pol. J. Puchta

TITLE OF PERSON IF OTHER THAN OWNER Wiss. Stiftungs-stand Adm. Stiftungsvorstand

ADDRESS OF PERSON SIGNING Eichenstr.1,69483 Wald-michelbach Eichenweg 12a, 69198 Schries-heim

SIGNATURE _____ DATE 29.02.2000

UNITED STATES PATENT APPLICATION

OF

JAN-HEINER KUPPER

ALEXANDER BURKLE

LEON VAN GOOL

AND

HARALD ZUR HAUSEN

FOR

MAMMAL WITH INHIBITION OF THE POLY (ADP RIBOSE) POLYMERASE AND METHOD FOR USING SAME TO IDENTIFY CANCERIGENIC AGENTS

INTELLECTUAL PROPERTY/TECHNOLOGY LAW
P.O. Box 14329 • Research Triangle Park, NC 27709

K 2564

A Method for Identifying Cancerigenic Agents Using a Mammal With Inhibition of the Poly(ADP Ribose)Polymerase

The present invention relates to a process for identifying cancerogenic agents.

A plurality of formerly unknown substances having cancerogenic danger potential and increasing in number because of constant new developments, occur in foodstuffs, cosmetics, textiles, materials, chemicals as well as other artificial products, but also in nature. In addition, physical agents (e.g. X-rays, U.V. radiation) can also cause cancer. Various in vivo and in vitro tests have been carried out so far to identify these substances and evaluate their cancerogenic potential.

The wide-spread Ames' test, which is also referred to as Salmonella typhimurium test, is based on the mutagenicity of substances in bacteria (Clonfero et al., Med. Lav. 81, pp. 3-10 (1990)). A likewise known in vitro test is the SOS chromotest (Quillardet et al., Mutat. Res. 297, pp. 235-279 (1993)) which is based on the induction of the bacterial SOS system by genotoxic agents. Both tests are comparable as regards their sensitivity but include the fundamental drawback that the genotoxic effects of substances in bacteria and higher organisms may differ and thus the results cannot be applied to the mammalian organism. Therefore the micronucleus test (Miller et al., Environ. Mol. Mutagen, 26, pp. 240-247 (1995), the single-cell gel test (SCG test, also referred to as cometary assay) and the test for sister chromosome exchange (Hartmann et al., Mutat. Res. 346, pp. 49-56 (1995) were developed few years ago. They are all based on eukaryotic cell systems.

For investigating the mutagenic effect of substances or physical agents in living organisms (in vivo), assays were developed which are based on the mutation of bacterial reporter genes (LacI or lac Z gene) which were inserted as transgene in mice (Gossen et al.,

Mutat. Res. 307, pp. 451-459 (1994). As a result, what is called the muta mouse and the big blue mouse were created.

Since the development of tumors is a process which comprises several factors and has not yet been elucidated in every detail, processes which only analyze individual aspects (e.g. mutation generation) of the tumor formation are insufficient. Therefore, the carcinogenicity of a substance or physical agent cannot be ruled out a priori in the case of a negative result. Furthermore, the bacterial systems usually used for the genotoxicity detection of chemical and physical agents can only be applied in limited fashion to higher organisms. Therefore, it must be stated that according to the current state of the art only the tumor formation as such is a safe parameter to determine the cancerogenic danger potential of a chemical or physical agent. For this reason, direct carcinogenicity tests using rodents had been introduced many years ago. However, in connection with these tests prescribed for the approval of new substances in many countries it is often necessary to apply very high doses of the corresponding substance so as not to obtain false negative results. Thus, the relevant objection was raised to the effect that the obtained positive results are not triggered by a real carcinogenicity of the substances but that only a non-specific stimulation of cell division is given which is due to overdosage. Mutations and, as a result, cancer would only form because of this mitogenic activity, so that these test which provide false positive results do not yield reliable results on the carcinogenicity of substances.

Therefore, it is the object of the present invention to provide a process by means of which it is possible to reliably identify cancerogenic agents.

This object is achieved by the subject matters defined in the claims.

The process according to the invention is carried out using a mammal, preferably a rodent, particularly preferably using a mouse, with DNA repair disturbance. The DNA repair disturbance is based on the trans-dominant inhibition of poly(ADP ribose)polymerase (abbreviated as PARP), which is an enzyme

involved in DNA repair processes. The inhibition of PARP is preferably based on the expression of a dominant negative mutant of poly(ADP ribose)polymerase, preferably the transgenic expression of such a mutant in a mammal, so that a transgenic animal is produced which is also a subject matter of the present invention. PARP has a DNA binding domain (abbreviated as DBD) which enables the binding to DNA strand breaks and results in an enzyme activity of PARP so as to enable repair of the strand breaks. However, a deletion is present in the dominant negative PARP mutant, so that only the DNA binding domain of PARP is expressed. This effects an inhibition of the PARP enzyme function and thus the DNA repair. The expression of this PARP mutant has no influence on the cell division and cell vitality in the absence of genotoxic stress. However, if genotoxic (chemical or physical) agents are applied, the PARP inhibition will lead to a considerable increase in the sensitivity of the cells to these treatments. The presence of the PARP mutant then results in an increased genetic instability after cancerogen treatment, which is manifested in an increased recombination as well as intensified gene amplification. Furthermore, the disturbance of the PARP function results in an increased mutagenicity of genotoxic agents. Thus, the disturbance of the cellular PARP function results in an increased rate of various genetic changes (mutations, recombinations, gene amplification) upon treatment with a cancerogen. As a function of the nature of the cancerogenic agent and the kind of its application, these various genetic changes permit various ways of tumor formation (e.g. oncogene amplification, tumor suppressor gene mutation or combinations thereof).

The mammal, preferably the transgenic mouse, used in the process according to the invention, has advantageously a skin-specific expression of the dominant-negative PARP mutant, every other organ-specific expression being, of course, also possible. However, because of its good accessibility and controllability the skin is the preferred organ for examinations as to carcinogenesis. For controlling the transgene it is possible to use every promoter known to the person skilled in the art and permitting a tissue-specific expression, preferably in the skin. The cytokeratin-14 promoter is used preferably. It permits an expression in the basal cell layer which is active as regards cell division and out of

which skin tumors develop preferably (Vassar et al., Proc. Natl. Acad. Sci USA 86, pp.1563-1567 (1989)).

It is preferred to use, for the production of the transgenic mammal, a fragment which has the following arrangement (see figure 1):

- 1.946 kb AvaI fragment of the human cytokeratin promoter (Vassar et al., Proc. Natl. Acad. Sci. USA 86, pp. 1563-1567 (1989))
- 1.156 kb DBD fragment from position -29 up to the internal NlaIV site at 1127 of the human poly(ADP ribose)polymerase (Cherney et al., Proc. Natl. Acad. Sci. USA 84, pp. 8370-8374 (1987))
- 0.486 kb of the polyadenylation signal of the human cytokeratin promoter (Vassar et al., Proc. Natl. Acad. Sci. USA 86, pp. 1563-1567 (1989))

A vector containing this fragment (pKDinoDBD) was deposited with the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen [German-type collection of microorganisms and cell cultures], Braunschweig) under number DSM 11594 on June 11, 1997.

The transgenic mammal is produced according to the method generally described by Hogan et al. ("Manipulating the mouse embryo: A laboratory manual", Cold Spring Habor Laboratory, New York (1986)). The microinjection of a corresponding DNA fragment into inseminated mouse oocytes and the subsequent implantation into apparently pregnant females are particularly suited for this purpose. Descendants develop which contain the transgene and pass it on to their descendants (DBD line).

The process according to the invention is carried out in the form of an in vivo assay. Transgenic DBD line animals which are 10 to 15 weeks old are selected and acclimatized correspondingly for the test. 10 to 15 animals (female or male) are required for every treatment. Since an important target organ of the carcinogenesis studies is the skin, the potentially cancerogenic chemical agents are applied topically in each case in 50 to 200 μl solvent, e.g. water, physiological salt solution, acetone or ethanol, two times

a week. For the investigation of potentially carcinogenic physical agents, corresponding applications are also carried out two times a week. These treatments can last up to 20 weeks. In order to enable tumor growth, an additional period of up to 40 to 80 weeks is estimated following the last application. 5 to 50 µg of 7,12-dimethylbenzanthracene (DMBA) can be used in the same test set-up as positive control for the tumor formation. The corresponding solvent which had also been used for dissolving the test substance can be applied as negative control. During the entire test period, the animals are weighed at regular intervals and the site of application is examined. Developing papillomas and other skin tumors, respectively, are investigated macroscopically once a week and optionally measured. As soon as the tumors have reached a critical size (depends on the animal species, the position of the tumor and the national animal protection conditions), the animals are killed and tumor tissue is collected for the histological and molecular-biological characterization. In addition, primary cultures of the tumors can be prepared. The results of the carcinogenicity experiments are evaluated statistically. In this connection, it is possible to determine dose-effect relationships. For the further analysis, it is possible to determine differences in the tumor formation in the sexes and with respect to wild-type mice. Further details on in vivo carcinogenicity assays are found in Tennant et al., Environ. Health Perspect. 103, pp. 942-950 (1995).

As compared to all in vitro models which select individual processes of tumor formation (e.g. DNA damage, mutation production), an in vivo assay whose biological end is the tumor formation as such is more significant. As compared to the above described direct carcinogenicity model using rodents, a sensitivity gain can be obtained by the process according to the invention by using the transgenic mammal wit DNA repair disturbance caused by trans-dominant inhibition of PARP activity so as to reduce the problem of overdosage and production of false positive results. In contrast to the known transgenic mouse models, the process of the present invention bypasses the problem of preparation towards a given tumor formation. The PARP inhibition results in a fundamental DNA repair disturbance and, as a consequence, an increase in the genetic instability (mutation,

recombination, gene amplification rates) following a cancerogenic treatment, so that the tumor formation is then promoted in various ways.

The invention is further described by means of the figures.

Figure 1 shows the expression fragment from pKDinoDBD
K14-prom. = promoter of the human cytokeratin-14 gene
DBD = coding sequence of the DNA binding domain of human poly(ADP ribose)polymerase (EC 2.4.2.30)
p-A = polyadenylation signal of the human cytokeratin-14 gene.

The invention is described in more detail by means of the below examples.

EXAMPLE 1: Production of the transgenic mouse line DBD # 354

The plasmid pKDinoDBD (see figure 1) was cleaved by the restriction enzyme NotI. Following the separation of the restriction fragments on a 1 % agarose gel, a 3.6 kb long fragment which contained the expression cassette of pKDinoDBD, was isolated and prepared by means of a commercially available kit (e.g. "Gene Clean"(r); Dianova company, Hamburg, Germany) according to the manufacturer's instructions. This fragment was adjusted to a concentration of 2 ng/μl in 10 mM Tris-HCl (pH 7.6), 0.25 mM EDTA. F1 females from the crossing of mouse strains C57BL/6 x DBA2 were subjected to superovulation by giving them hormones. After the pairing with F1 males (also by crossing of C57BL/6 x DBA2), inseminated egg cells from the females were prepared and the above described DNA fragment was microinjected thereinto. The embryos were implanted into the uterine tubes of apparently pregnant NMRI mice (nurse mothers; previously paired with vasectomized males). The animals born after about 21 days were tested by means of DNA material from tail biopsies for the presence of the transgene. For this purpose, the technique of polymerase chain reaction (PCR) was used with the following primers from the coding sequence of human PARP (Cherney et al., Proc. Natl. Acad. Sci. USA 84, pp. 8370-8374 (1987)):

5'-ATG GCG GAG TCT TCG GAT AAG CTC TA-3' (primer 1, # 1-26)
5'-GCC AGG CGT GGC CGC CAC GGA GG-3' (primer 2, # 1110-1088)

22 PCR cycles were carried out with 200 ng genomic DNA each. In each case, denaturation was carried out at 95°C for 300 seconds, attachment was made at 60°C for 60 seconds and polymerization took place at 72°C for 120 seconds.

A positive female (earmark #354) was identified. Protein material was obtained from the tail biopsy of this animal and investigated for expression of the transgene by means of Western blot. In this connection, both the monoclonal anti-DBD antibody Cl110 (Lamarre et al., Biochim. Biophys. Acta 950, pp. 147-160 (1988)) and the anti-Fl1 rabbit serum directed against DBD (Küpper et al., J. Biol. Chem. 265, pp. 18721-18724 (1990)) were used. The DNA binding domain (DBD) of 45 kDa could be detected in the Western blot by means of both antibodies, so that the evidence for the expression of the transgene was furnished. The founder-DBD mouse #354 was paired with DBA2 males and the descendants were analyzed. The transgene is passed on to the descendants, so that the line DBD #354 is stably present.

EXAMPLE 2: Identification of the cancerogenic potential of five different chemicals

12-week-old animals of the mouse DBD line #354 described in Example 1 are acclimatized to the test site for three weeks. Female animals are kept in groups of 5 animals per cage, male animals are kept singly under specific pathogen-free (SPF) conditions. The animals are fed according to standard (#D10010 food from Research Diets, New Brunswick, New Jersey, U.S.A., and water ad libitum). 10 to 15 animals (male or female) are required for every treatment. The putatively carcinogenic chemicals to be tested are taken up in several dilution stages in physiological salt solution and acetone, respectively, and 100 µl of every dilution is applied topically in each case two times a week. 20 µg of 7,12-dimethylbenzanthracene (DMBA) in 100 µl acetone are used as positive control. Acetone is applied as negative control. The treatment is carried out for 15 weeks. The animals are weighed

weekly and the application sites are examined. 12 weeks after the end of the treatments, visible tumor growth can be expected in the group of positive control, the tumors rapidly (within 12 further weeks) increasing in size according to experience. Having reached a critical tumor size, the animals are killed by cervical dislocation each and the tumors are removed. As expected, no tumor growth is found in the group of animals treated with solvent even after 60 weeks.

As an alternative, what is called an initiation promotion protocol can be used. In principle, a very low dose of an initiating (usually DNA damaging) carcinogen is administered once in this case, followed by repeated applications of a tumor promoter which is not carcinogenic as such (Becker et al., Cancer Res. 56, pp. 3244-3249, 1996). Here, e.g. nitrosomethylurea (20 µmol in 100 µl acetone; applied topically once) is used as positive control. Seven days later, the treatment is continued with the tumor promoter tetradecanoyl-phorbol-acetate (TPA) two times a week for a period of 22 weeks (in each case 10 mmol in 100 µl acetone). Here, the negative controls are animals which in place of nitrosomethylurea were only given acetone, but then followed by the common TPA treatment. The chemicals to be tested for carcinogenicity are also applied in place of the nitrosomethylurea, again followed by the common TPA treatment. In the case of this protocol, visible tumor growth in the DHD line #354 mouse described in Example 1 has to be expected in the positive control after 9 weeks at the latest.

Claims

1) A process for identifying cancerogenic agents, wherein the potentially cancerogenic agents are administered to a mammal with a DNA repair disturbance caused by inhibiting the poly(ADP-ribose)polymerase.

2) The process according to claim 1, wherein the inhibition of the poly(ADP ribose)polymerase is caused by the expression of a dominant negative poly(ADP ribose)polymerase.

3) The process according to claim 1 or 2, wherein the inhibition of the poly(ADP ribose)polymerase is made by a transgenic operation.

4) The process according to any one of claims 1 to 3, wherein the mammal used is a transgenic mouse.

5) The process according to any one of claims 1 to 4, wherein the potentially cancerogenic agents are administered by topical application.

6) The process according to any one of claims 1 to 5, wherein the mammal expresses transgenically the DNA construct shown in figure 1.

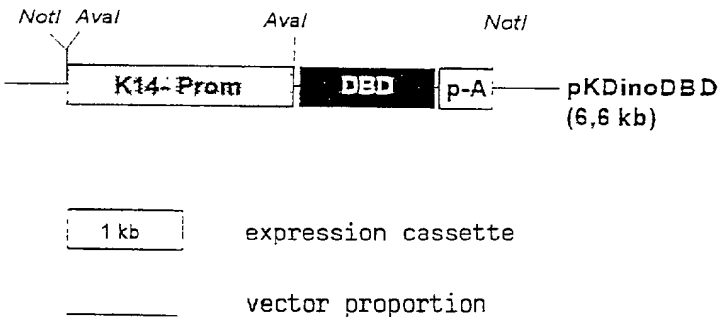
7) Use of a mammal with DNA repair disturbance caused by inhibiting poly(ADP ribose)polymerase for carrying out the process according to any one of claims 1 to 6.

8) Use according to claim 7, wherein the inhibition of the poly(ADP ribose)polymerase is caused by expression of a dominant negative poly(ADP ribose)polymerase.

9) Use according to claim 7 or 8, wherein the mammal is a transgenic mouse.

Abstract of the Disclosure

The present application relates to a process for identifying cancerogenic agents, wherein the potentially cancerogenic agents are administered to a mammal with a DNA repair disturbance caused by inhibiting the poly(ADP ribose)polymerase.


NotI AvaI
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1 kb
expression cassette
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Fig. 1

P245/K2771

Docket No. 4121-115

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As the below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name.

I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **MAMMAL WITH INHIBITION OF THE POLY (ADP RIBOSE) POLYMERASE AND METHOD FOR USING SAME TO IDENTIFY CANCERIGENIC AGENTS,** the specification of which was filed on December 23, 1999, based on International Patent Application No. PCT/DE98/01797 claiming priority of German Patent Application No. 197 26 824.2.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

197 26 824.2	24 June 1997	
(Application Number)	(Filing Date)	(Status-Patented, Pending, abandoned)

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Docket No. 4121-115

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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